## REMARKS

As a preliminary matter, Applicants respectfully request that the Attorney Docket No. for the above-referenced application be corrected from 9725-005 to 289550-122US2.

Applicants gratefully acknowledge that Applicants' Amendment filed February 4, 2004 has been entered.

### I. Amendments to the Specification:

The specification has been amended to correct minor typographical or clerical errors. No new matter has been added by way of these amendments.

### II. Amendments to the Claims:

Claims 10-13, 18-20 and 22-26 were pending in this application.

Claims 10, 13, 18-20, 22, 23 and 26 have been amended. Claims 27-39 have been newly added. No new matter has been introduced into the application by way of these amendments to the claims. Claims 10, 13, 19, 20, 22 and 23 have been amended to more clearly claim the present invention. These amendments raise no issue of new matter. Support for the amendment to claim 18 can be found *inter alia* at page 10, lines 32-35, page 11, lines 23-25 and page 28, lines 20-24. Support for newly added claims 27–39 can be found *inter alia* at page 19, lines 21-32, and in the claims as originally filed. Applicants would like to clarify that new claim 37, directed to a composition, is broader than claim 23 directed to a protein that is part of a pharmaceutical composition. In other words, claim 37 encompasses pharmaceutical compositions in addition to other compositions.

Accordingly, upon entry of the instant amendment, claims 10-13, 18-20, and 22-39 are pending in this application.

## III. Amendments to the Drawings:

Figures 15 A-B and 16 A-D have been amended to include SEQ ID NOs. This amendment raises no issue of new matter as the Sequence Listing under 37 C.F.R. § 1.821 containing these SEQ ID NOs was submitted to the USPTO on August 14, 2001.

## IV. Abstract:

The Examiner has alleged that the application does not contain an abstract of the disclosure as required by 37 C.F.R. § 1.72(b) (*see*, pp. 8-9 of Office Action). In reply, Applicants note that the Application was filed with an Abstract in compliance with 37 C.F.R. § 1.72(b) (*see*, p.136 of the Application as filed). Furthermore, the submitted Abstract was published with the application on May 23, 2002, in United States Patent Application Publication US 2002/0061549.

## V. Rejections Under 35 U.S.C. § 112, first paragraph:

Claims 10-13 and 22-26 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled by the specification. The Examiner stated that the specification is enabling for "an isolated, stabilized protein, comprising isolating a specific polypeptide, selecting one or more tyrosine residue pairs in a polypeptide chain, cross-linking the tyrosine residue pair(s) under defined conditions..." However, the Examiner stated that the specification is not enabling for "any isolated protein comprising a di-tyrosine cross-link by [*sic*] wherein at least one tyrosine of a di-tyrosine cross-link originates from a point mutation." (*see*, p. 2 of Office Action).

Specifically, the Examiner alleged that Applicants have no basis for 'point mutation' in their specification (*see*, p. 4 of Office Action). In reply, Applicants refer the Examiner to the following sections of the Application as filed: p. 28, ll. 20-24; p. 32, ll. 11-25; p. 33, ll. 11-23; p. 57, ll. 15-21; p. 111, ll. 4-26; and the paragraph bridging pp. 122

and 123. These sections of the application clearly provide more than adequate basis for point mutations, including point mutations of any residue to tyrosine. For example, the specification states:

> Moreover, ***point mutations*** to tyrosine may be introduced at positions where the tyrosyl side-chains will react with each other to form a bond that causes the least distortion to structure and function; these positions are identified as described in detail below. Thereby, the overall structure and functionality of the polypeptide or polypeptide complex is maintained. (emphasis added). (*see*, p. 32, ll. 21-25).

The specification also states:

> When at a selected residue of either, or any, polypeptide(s) the reactive tyrosyl side-chain is not already present, a ***point mutation*** may be introduced, for example, but not limited to, by using molecular biological methods to introduce such a point mutation into the cDNA of the gene directing its expression, such that a reactive side-chain is present and available for the reaction. (emphasis added). (*see*, p. 33, ll. 19-23).

In addition, in the section entitled "Introduction of Point Mutations to Phenylalanine," the specification states:

> One of the codons of every tyrosine residue pair that may react with each other and cause undesirable structural and/or functional distortions is preferably ***point mutated*** to codons that direct the expression of phenylalanine.
>
> ***Point mutations*** can be introduced into the DNA encoding the polypeptide, or one, any, both, several, or all of the polypepitdes of a complex by any method known in the art, such as oligonucleotide mediated site-directed mutagenesis. Such methods may utilize oligonucleotides that are homologous to the flanking sequences of such codons, but that encode tyrosine at the selected site or sites. With these oligonucleotides, DNA fragments containing the ***point mutation or point mutations*** are amplified and inserted into the gene or genes, for example, by subcloning. One example of such methods is the application of the QuikChange™ Site-Directed Mutagenesis Kit (Stratagene, Catalog #200518); this kit uses the Pfu enzyme having non-strand-displacing action in any double stranded plasmid mutation in PCR reactions. Other methods may utilize other enzymes such as DNA polymerases, or fragments and/or analogs thereof.
>
> The plasmid or plasmids containing the point mutation or point mutations are, for example, transformed into bacteria for expansion, and

> the DNA is prepared as described above. The isolated, expanded, and prepared DNA may be examined to verify that it encodes the polypeptide or polypeptides of the complex, and the correct mutation or mutations were achieved. This may, for example, be verified by direct DNA sequencing, DNA hybridization techniques, or any other method known in the art. (emphasis added). (*see*, p. 54, l. 33 to p. 55, ll. 1-17).

And furthermore, in the section entitled, "Point Mutation to Tyrosine and Gene Product Purification" the specification states:

> The codons of the residues identified as a suitable pair to which the cross-link should be directed, as described above, and selected for a particular embodiment of the instant invention, are ***point mutated*** such that the resultant residue pairs direct the expression of tyrosyl side-chains. ***Point mutations*** are introduced as described above. (emphasis added). (emphasis added). (*see*, p. 57, ll. 17-20).

Thus, in contrast to the Examiner's position, Applicants respectfully aver that there is more than adequate basis in the specification for "point mutations."

The Examiner has also alleged that genetic expression of tyrosyl-tyrosyl cross-linked proteins remains unknown and therefore claims to recombinantly produced cross-linked protein(s) or that obtained by point mutation, or compositions thereof are not enabled (*see*, p. 4 of Office action). In reply, Applicants call the Examiner's attention to the language of the claims. Independent claims 10, 18 and 30 do not recite "genetic expression" of tyrosyl cross-linked proteins. Applicants previously amended claim 10 (Amendment filed February 4, 2004) to remove the phrase "introduced by genetic engineering." Applicants respectfully point out that the Examiner may be incorrectly importing a limitation that is not in the claim into the language of the claims. Independent claim 10 is directed to an isolated protein comprising at least one di-tyrosine cross-link, wherein at least one tyrosine of a di-tyrosine cross-link originates from a point mutation to tyrosine, and wherein the di-tyrosine cross-linked protein retains at least one function displayed by the protein in the absence of di-tyrosine cross-linking. Nowhere, in this claim is there a recitation of genetic expression of tyrosine

cross-linked proteins. In the absence of language in the claims indicating "genetic expression" of di-tyrosyl cross-linked proteins, Applicants respectfully aver that this ground of rejection is improper.

Applicants respectfully contend that the application as filed adequately enables the claimed invention. The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *United States v. Telectronics, Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). The application as filed is enabling with respect to the currently pending claims because there is considerable direction and guidance in the specification to achieve the claimed invention. As discussed above there are several sections of the specification that teach introducing a di-tyrosine cross-link where at least one tyrosine originates from a point mutation (*see*, for example, p. 28, ll. 20-24; p. 32, ll. 11-25; p. 33, ll. 11-23; p. 57, ll. 15-21; p. 111, ll. 4-26; and the paragraph bridging pp. 122 and 123). In addition, there is explicit guidance in the examples for constructing point mutations to form a di-tyrosine bond (*see*, Examples I-III). For example in Example I it is stated:

> At the residues of the pair selected, as described above, amino acid substitutions are introduced by point mutation, so far as tyrosine is not already present at the selected residues of the pair in the sequences of the heavy and light chains of the Fv fragment to be stabilized. Point mutations are introduced by using the QuikChange™ Site-Directed Mutagenesis Kit (see above). (*see*, p. 101, ll. 16-21)

In Example II, which relates to stabilization of *Candida albicans* Lipase B (CALB), it is stated:

> At the residues of the pair selected, as described above, amino acid substitutions are introduced by point mutation, so far as tyrosine is not already present at the selected residues, using forward primer for M1 together with Primer B, and forward and reverse primers for M2 and M3, as described in FIG. 15B. Point mutations are introduced by using the QuikChange™ Site-Directed Mutagenesis Kit (see above). p. 111, ll. 20-26).

Also, in Example III, relating to stabilization of Subtilisin E, it is stated:

> According to the final selection of residue pairs (Tables 15 and 16, FIG. 16D), PCR is used to introduce point mutations to tyrosine, and Primers A and B) to introduce a poly-histidine tag to the polypeptide. Point mutations are introduced by PCR using the QuikChange™ Site-Directed Mutagenesis Kit (Stratagene, 1998 Catalog # 200518). The 5' primer (FIG. 16D, Primer A) creates an NdeI site, and the 3' primer (FIG. 16D, Primer B) creates a BamH1 site. (para. bridging pp. 122 and 123).

Such point mutated genes are expressed (*e.g.*, p. 101, ll. 24-35 and p. 102, ll. 3-21), and the point mutant proteins are subsequently isolated/purified (*e.g.*, p. 28, ll. 7-24; p. 57, l. 24 to p. 58, l. 8), and thereafter subjected to cross-linking conditions that favor the chemical reactions that leads to the formation of covalent bonds between tyrosyl residues that are in close proximity (di-tyrosine bonds) (*e.g.*, p. 55, l. 32 to p. 57, l. 11; p. 57, l. 24 to p. 58, l. 8; p. 102, l. 25 to p. 103 l. 11; and p. 113, ll. 10-33).

Applicants respectfully contend that the guidance provided in the application would allow one of ordinary skill in the art to make and use the claimed invention. The ordinary skilled artisan would have routinely been able to make point mutations in any protein (as the Application makes clear, kits such as Quik Change™ Site-Directed Mutagenesis kit from Stratagene were available to the artisan) and to chemically crosslink proteins (as evidenced by the Examiner-cited references, Aeschbach *et al.* and Brown *et al.*). Taken together, Applicants respectfully assert that claims 10-13 and 22-26 were fully enabled at the time of the filing of the instant Application.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

## VI. Rejections Under 35 U.S.C. § 112, second paragraph:

Claims 13, 20 and 22-26 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite (*see*, p. 5 of Office Action).

In reply, applicants traverse the rejection, but in the interest of accelerated prosecution, have amended claims 13, 20, 22 and 23.

Claim 13 has been amended to more particularly point out the presently claimed invention. In particular, the claim has been amended to recite, in relevant part, "wherein the protein *is* an enzyme, an antibody or a fragment thereof."

Claim 20 has been amended to more particularly point out the presently claimed invention. In particular, the claim has been amended to recite, in relevant part, "wherein ***the di-tyrosine*** crosslinking is catalyzed by a catalyst selected from the group consisting of polyhistidine, Gly-Gly-His, a metalloporphyrin, a peroxidase, or any combination thereof."

Claim 22 has been amended to more particularly point out the presently claimed invention and recites, in relevant part, "wherein the protein *is* a hormone, a receptor, a growth factor, an enzyme, or an antibody."

Claim 23 has also been amended to more particularly point out the presently claimed invention and recites, in relevant part, "wherein the protein is part of a pharmaceutical composition." The Examiner alleged that claims to pharmaceutical compositions are not enabled by the application. Applicants respectfully disagree. There is sufficient support in the specification for pharmaceutical compositions (*see*, for example, the sections entitled "Pharmaceutical Compositions" and "Considerations for Pharmaceutical Composition" at pp. 65-67). Any protein, including antibodies, used for the treatment or prevention of any disease, or complication from any disease, can be stabilized using the methods taught by Applicants. This would be readily understood by any person of ordinary skill in the art upon reviewing the instant application.

In view of the foregoing remarks, Applicant respectfully requests the Examiner to reconsider and withdraw this ground of rejection.

VII. Rejections Under 35 U.S.C. §102(b):

(a) *Aeschbach et al.*

Claims 18-19 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Aeschbach *et al.* (1976), BBA 439, 292-302 (AA – IDS) (*see*, p. 6 of Office Action). The Examiner stated that Aeschbach *et al.* teach a method for formation of di-tyrosine cross-links in proteins, the hormone insulin for example, by oxidation using hydrogen peroxide. The Examiner takes the position that the reference teaches all of the elements of the claims.

Amended claim 18 is directed to a method for making a stabilized protein comprising selecting one or more residue pairs in a polypeptide chain or chains for di-tyrosine cross-linking, ***wherein at least one tyrosine of a di-tyrosine cross-link originates from a point mutation to tyrosine;*** mutating at least one of the selected residues for cross-linking to tyrosine; and cross-linking the residues pairs; wherein the di-tyrosine cross-linked protein retains at least one function displayed by the protein in the absence of di-tyrosine cross-linking.

Aeschbach *et al.* do not disclose "wherein at least one tyrosine of a di-tyrosine cross-link originates from a point mutation to tyrosine" as required by claim 18.

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

Because Aeschbach does not disclose each and every element of claims 18 and 19, Applicants respectfully assert that Aeschbach *et al.* do not anticipate claims 18-19.

Accordingly, Applicants request the Examiner to reconsider and withdraw this ground of rejection.

(b) *Brown et al.*

The Examiner rejected claims 10-13, 18-20 & 22 under 35 U.S.C. § 102(b) as being anticipated by Brown *et al.* [*Biochemistry*, 1998, 37: 4397-4406, AD – IDS] (*see*, p. 7 of Office Action). Specifically, the Examiner took the position that Brown *et al.* suggest that residues can be changed with little or no effect on the binding affinity or the protein structure. The Examiner opined that this is indicative of the fact the function of the protein is unaffected.

In reply, Applicants traverse the rejection and assert that the pending claims are not anticipated by the Brown *et al.* reference because it does not teach each and every element of the claimed invention. Specifically, this reference does not teach a di-tyrosine cross-linked protein, wherein at least one tyrosine of the di-tyrosine cross-link originates from a point mutation to tyrosine, and wherein the ***di-tyrosine cross-linked protein retains at least one function displayed by the protein in the absence of di-tyrosine cross-linking***, as required by all the currently pending claims.

In contrast to the Examiner's position, Applicants respectfully assert that the section of the Brown *et al.* reference relied upon by the Examiner (p. 4402, col. 1 and 2, ll. 1-10) does not disclose that di-tyrosine cross-linked GGH-ecotin D137Y protein retains at least one function of an ecotin D137Y protein in the absence of cross-linking. Brown *et al.* disclose that ecotin is a serine protease inhibitor that binds to a serine protease as a homodimer to inhibit its function. Brown *et al.* does not address whether GGH-ecotin D137Y or di-tyrosine cross-linked GGH-ecotin D137Y can bind a serine protease, or whether these ***cross-linked*** proteins can inhibit the function of a serine protease. The language the Examiner refers to in the Brown *et al.* reference (p. 4402, col. 1 and 2, ll. 1-10) describes the mutant's retained function, which is to form a homodimer ***prior*** to cross-linking. It does not describe the retained function of ***cross-linked*** GGH-ecotin D137Y homodimer to bind a serine protease (*e.g.*, trypsin) and/or

inhibit its proteolytic activity/function; nowhere in Brown *et al.* is this element disclosed.

In fact, the teachings in the Brown *et al.* reference strongly suggest that GGH-ecotin D137Y does not have serine protease inhibition activity/function after cross-linking. This reference clearly discloses that unlike the uncross-linked ecotin homodimer, cross-linked GGH-ecotin does not bind to trypsin as a dimer.

> In the presence of trypsin, GGH-Ni(II)-ecotin is cross-linked to yield species with apparent molecular masses of 32kDA, corresponding to an ecotin-ecotin dimer, and 40kDa, the expected mass for an ecotin-trypsin heterodimer (Figure 3, lane 2). The reaction was perfomed in the presence of 250mM tyrosine to ensure that nonspecific cross-linked products were not formed. Immunoblotting with antibodies against ecotin confirmed the identity of the two species. ***Interestingly, no ecotin2-trypsin2 complex was observed by SDS-PAGE.*** (emphasis added). (p. 4401 under "GGH-Ni(II)-Ecotin Can Be Cross-Linked to Its Protease Targets", first paragraph).

The above findings can be explained by cross-linked GGH-ecotin's inability to bind to serine proteases, such as trypsin, in the same manner as uncross-linked ecotin. This suggests that GGH-ecotin looses serine protease inhibition activity/function by cross-linking. Given that this reference describes functional equivalence of the point mutant and wild type ecotin, to the limited extent it was examined (homodimer formation), it is likely that the di-tyrosine cross-linked GGH-Ecotin D137Y dimer also looses its ability to bind serine proteases (*e.g.*, trypsin), and that it therefore also looses serine protease inhibition activity/function by cross-linking. In any event, nowhere in Brown *et al.* is there any teaching or suggestion that ***cross-linked*** GGH-Ecotin D137Y retains at least one function displayed by uncross-linked GGH-ecotin.

Independent claims 10, 18 and 30, and the claims depending therefrom, require that at least one tyrosine of a di-tyrosine cross-link originates from a point mutation to tyrosine, and that *the di-tyrosine cross-linked protein retains at least one function displayed by the protein in the absence of di-tyrosine cross-linking*. Because Brown *et al.* do not teach

each and every element of Applicants' claims, Applicants contend that Brown *et al.* do not anticipate Applicants' claimed invention.

Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

## CONCLUSION

Applicants respectfully submit that the application and claims are now in condition for allowance. If the Examiner believes that further discussion would be helpful, he is respectfully requested to telephone the undersigned attorney at (212) 937-7233 and is assured of full cooperation to advance the application to allowance.

No fees are believed to be due in connection with this filing; however, if any fees are due, the Commissioner is hereby authorized to charge any fee(s) that may be necessary in this application to Deposit Account No. 08-0219, Order No. 289550-122US2.

Respectfully submitted,

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